# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities




WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
## Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter

# Prevention and Control of American Foulbrood in South America with Essential Oils: Review

*Sandra Rosa Fuselli, Pablo Gimenez Martinez, Giselle Fuentes, Rosa María Alonso-Salces and Matías Maggi*

## Abstract

American foulbrood (AFB) is the most severe bacterial disease that affects honey bees, having a nearly cosmopolitan distribution. AFB's causative agent is *Paenibacillus larvae*. AFB kills infected honey bee larvae; however, it eventually leads to the collapse of the entire colony when left untreated. The infection takes place by the ingestion of the spores with the food provided by adult worker bees to the larvae. In South America (SA) the disease was first described in 1989 in Argentina, constituting the first sanitary challenge for beekeepers to overcome. Prevention and control measures of AFB in SA countries generally include vigilance for early diagnosis, isolation of apiaries with cases of AFB, and multiplication of healthy colonies with hygienic queens, among others. The extensive use of tetracycline hydrochloride in Argentina has led to the development of resistant *P. larvae* isolates. In this context, the development of alternative and effective methods for the control and prevention of AFB disease is crucial. Currently, alternative strategies for the prevention and treatment of AFB are being studied, mainly based on essential oils.

**Keywords:** *Paenibacillus larvae*, essential oils, quorum sensing, American foulbrood, *Apis mellifera*

## 1. Introduction

Along with wild bees, honeybees are the most important crop pollinators [1, 2]. *Apis mellifera* pollinates 77% of the plants responsible for producing food resources which sustain the global human population [1]. Since 1998, individual beekeepers have reported the unusual weakening and mortality of colonies, particularly in France, Belgium, Switzerland, Germany, the United Kingdom, the Netherlands, Italy, Spain, and North America [3, 4]. Most scientists agree that there is no single explanation for the extensive colony losses, but that interactions between different stressors are likely involved [5].

American foulbrood (AFB) is the most severe bacterial disease that affects honey bees, having a nearly cosmopolitan distribution (**Figure 1**) [6]. AFB only kills

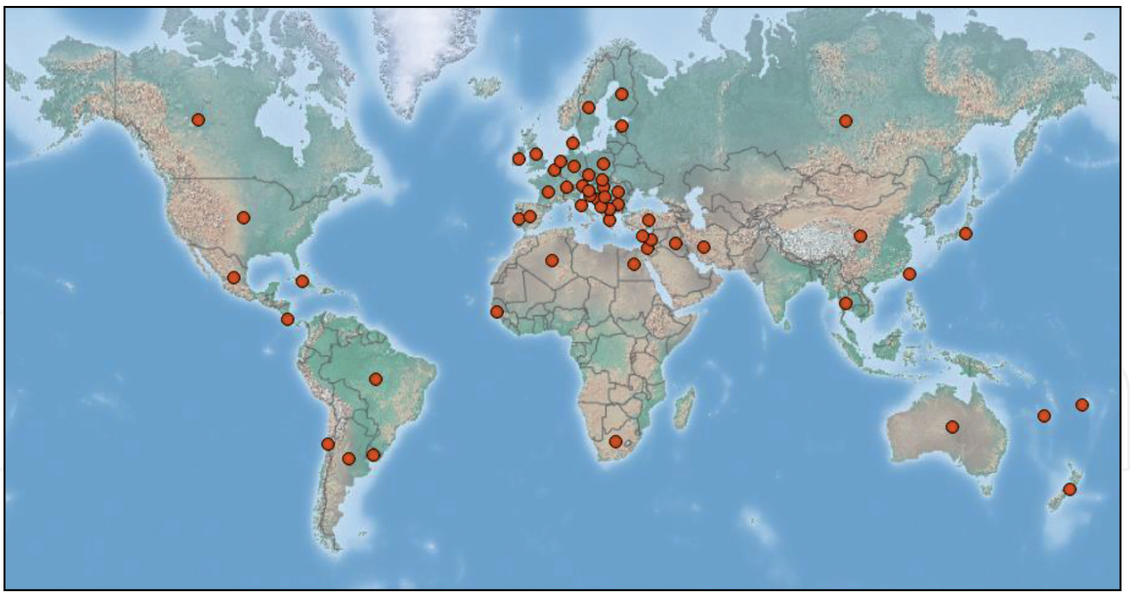


**Figure 1.**
*Distribution of American foulbrood. (https://www.cabi.org/isc/datasheet/78183).*

infected honey bee larvae; however, it eventually leads to the collapse of the entire colony when left untreated. AFB is considered to be very contagious; therefore, it is a notifiable disease in most countries [7]. AFB's causative agent is *Paenibacillus larvae*, which is a flagellated gram-positive bacterium, whose main characteristic is the formation of highly resistant endospores. This pathogen affects the breeding during the larval or pupal stages [8]; its spores being the infectious form. Honey bee larvae are more susceptible to infection during the first 36 h after egg hatching [9], indeed only 10 spores are required to make a larva of less than 24 h old ill [10]. However, at later larval developmental stages, spore doses needed to successfully infect a larva are too high to occur under natural conditions [11]. The infection takes place by the ingestion of the spores with the food provided by adult worker bees (nurses) to the larvae [12]. The spores after germinating in the midgut of the larvae proliferate for several days. After this, *P. larvae* reaches the peritrophic matrix, penetrates the epidermal cells, produces septicemia causing death of the larva. Finally, dead larvae are digested by vegetative bacterial cells and converted to dry flakes containing millions of spores of *P. larvae* [12, 13]. The most evident symptoms of AFB are the irregular coating of the offspring, which show cells with cap and uncovered irregularly dispersed through the frames of the offspring; dark, sunken, and often perforated caps emitting a characteristic AFB odor; remnants of brown glue from the dead larvae forming a characteristic cord thread when removed with a wooden stick or an inlay; and a hard scale of larval residues at the bottom of the cell. The traditional diagnosis is made based on the observation of these clinical symptoms in the hive and in the microbial culture of material from infected colonies [14].

AFB was first described in South America (SA) in Argentina, in 1989, constituting the first sanitary challenge for beekeepers to overcome. It was hypothesized that the entrance of *P. larvae*, into the country was through bees imported from the USA [15]. AFB quickly spread to most important beekeeping centers of the country [16], with incidences as high as 30% in some geographic areas [17]. At least 30–45% of the colonies were lost due to AFB during those years (Eguaras, unpublished data). AFB was extended to Chile in 2002 and was controlled. New outbreaks were detected in 2005 in different regions [18] (**Table 1**).

In some countries the use of antibiotics, particularly tetracycline hydrochloride (OTC) [6, 12], is the most common method for prevention and treatment of

| | | |
|---|---|---|
| Argentina | Restricted distribution | [15, 84] |
| Bolivia | No information available | [84] |
| Brazil | Present | [84] |
| Chile | Present | [84] |
| Colombia | Disease never reported | [84] |
| Ecuador | Disease never reported | [84] |
| French Guiana | Disease never reported | [84] |
| Peru | Disease not reported | [84] |
| Uruguay | Present | [84] |
| Venezuela | Disease never reported | [84] |

**Table 1.**
*Distribution of P. larvae in South America.*

infected colonies. However, in most European countries the use of antibiotics is banned, since their use is known to generate several problems including the presence of chemical residues in the beehive products (honey, pollen and wax), which eventually may even affect consumer health. Moreover, antibiotic application can affect life of bees and can increase the risk of occurrence of resistant strains [19]. To date, the presence of OTC resistant strains has been reported in Argentina, United States, Italy, New Zealand and United Kingdom [16, 20].

Prevention and control measures of AFB in SA countries generally include vigilance for early diagnosis, isolation of apiaries with cases of AFB, and multiplication of healthy colonies with hygienic queens, among others [21]. Brazilian, Chilean, and Uruguayan authorities specifically recommend the burning of colonies containing clinical signs of the disease in order to control the outbreaks [21]. The use of antibiotics in SA is not allowed, except in Argentina [18]. The extensive use of OTC in this country has led to the development of resistant *P. larvae* isolates [16], which is a major concern for Argentine beekeepers. In contrast, in Uruguay and Chile, where their use is not authorized, no resistant strains have been detected [22]. The endospore resistance of *P. larvae* is an important problem in the control and prevention of AFB because these individuals can survive for more than 35 years in honey and/or beekeeping material and is resistant to high temperatures as well as to the most used disinfectants [10]. Most treatments are based on the use of broad spectrum antibiotics, which, in most cases, have been used continuously and excessively. In fact, different antibiotics, such as sulfathiazole and OTC, are able to inhibit the growth of *P. larvae*, but its use and abuse during the last years has led to the appearance of resistant strains and residues that contaminate the products of the hive. For these reasons, the use of antibiotics for the treatment and prevention of AFB is prohibited in several countries, and the affected colonies must be destroyed [23].

In this context, the development of alternative and effective methods for the control and prevention of AFB disease is crucial. These methods may consider the evidence of the bacteria-resistant phenomenon and meet the strict EU standards, as well as current trends in green consumption [24, 25]. Currently, alternative strategies for the prevention and treatment of AFB are being studied, mainly based on essential oils [25–27], probiotics and propolis [28].

### 1.1 Essential oils

In light of developments in the scientific field, the medicinal properties of plants have received great interest due to their low toxicity, pharmacological activities

and economic viability [29]. These studies have focused on the benefits of phytochemicals extracted from plants and their effect on human health. The additives naturally obtained from plants can be individual compounds, groups of compounds or essential oils (EOs). In recent times, there has been an increase in the interest of the food industry in natural compounds, either by direct addition or by its use in synergy with other compounds. It has been reported that the direct addition of essential oils and extracts of aromatic plants to food products exerts its antioxidant or antimicrobial effect [30].

Plants and other natural sources can provide a wide variety of complex and structurally diverse compounds. Plant extracts and essential oils have antifungal, antibacterial and antiviral properties and have been evaluated worldwide as potential sources of new antimicrobial compounds, agents that promote food preservation and alternatives to treat infectious diseases [31, 32]. It has been reported that essential oils possess significant antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal activities [33, 34]. Therefore, essential oils can serve as powerful tools to reduce bacterial resistance [33]. Oily aromatic liquids called essential oils (also called volatile oils) are obtained from plant materials (leaves, buds, fruits, flowers, herbs, branches, bark, wood, roots and seeds).

Being natural mixtures of very complex nature, the essential oils can consist of approximately 20–60 components at quite different concentrations. Essential oils are characterized by two or three main components that are present in fairly high concentrations (20–70%) compared to other components that are present in trace amounts. The amount of different components of essential oils varies between different parts of plants and different plant species since they are derived chemically from terpenes and their oxygenated derivatives, i.e., terpenoids which are esters of aromatic and aliphatic acid, and phenolic compounds. An important characteristic of essential oils and their components is their hydrophobicity, which allows them to interact with the lipids present in the cell membrane of bacteria and mitochondria, making them more permeable by altering their cellular structures. This eventually results in the death of bacterial cells due to the leakage of critical molecules and ions from the bacterial cell. Some compounds modulate drug resistance by targeting efflux mechanisms in several species of gram-negative bacteria [35]. An important function of essential oils in nature is the protection of plants by acting as antifungal, antibacterial, antiviral and insecticidal agents and also protection against herbivores by reducing the appetite of herbivores for plants with such properties. Health Services and Human Services Public Health Services have recognized essential oils as safe substances, and some of them contain compounds that can be used as antibacterial additives [33]. The efficacy of EOs has been reported in several studies against pathogens and food contaminants [36], suggesting their applications in the food industry [34, 37]. Several EOs have been evaluated for the *in vitro* and *in vivo* control of *P. larvae* (**Table 2**), as well as their acute oral toxicity to *Apis mellifera* (**Table 3**).

### *1.1.1 In vitro assays to control P. larvae*

EOs from *Achyrocline satureioides*, *Carum carvi*, *Cinnamomum* spp., *Cinnamomum zeylanicum*, *Citrus paradise*, *Cuminum cyminum*, *Cymbopogon citratus*, *Eucalyptus cinerea*, *Melaleuca alternifolia*, *Mentha piperita*, *Minthostachys verticillata*, *Origanum majorana*, *Origanum vulgare*, *Polygonum bistorta*, *Salvia officinalis*, *Salvia sclarea*, *Syzygium aromaticum*, *Tagetes minuta*, *Thymus vulgaris*, *Verbena*, *Pimenta dioica* (L.) Merr., *Litsea cubeba* Pers., *Trachyspermum ammi* L., *Mentha arvensis* L., *Mentha spicata* L., *Illicium verum* Hook.f, *Myristica fragrans* Gronov., *Cinnamomum camphora* (L.) J. Presl., *Ocimum tenuiflorum* L., *Daucus carota* L., *Zingiber officinale* Rosc., and

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Acantholippia seriphioides* A. Gray | Broth macrodilution | Inhibitory | | 236 mg/L | | [26] |
| | Broth macrodilution | Inhibitory | | 300 mg/L | | [41] |
| *Achyrocline satureioides* Lam. | Agar diffusion | Inhibitory | 10 μl | | | [33] |
| *Artemisia absinthium* L. | Broth microdilution | Inhibitory | | 416 mg/L | 647 mg/L | [42] |
| *Artemisia annua* L. | Broth microdilution | Inhibitory | | 402 mg/L | 624 mg/L | [42] |
| *Aloysia polystachia* Griseb. | Broth microdilution | Inhibitory | | 700–800 mg/L | 900 mg/L | [42] |
| *Carapa guianensis* Aubl. | Broth microdilution | Inhibitory | | 25% (v/v) | | [27] |
| *Carum carvi* L. | Agar diffusion | Inhibitory | 5, 10 μl | | | [35] |
| *Chamomilla recutita* L. | Agar diffusion | Non-inhibitory | 5, 10 μl | | | [35] |
| *Cinnamomum aromaticum* L. | Agar diffusion | Inhibitory | 10 μl | | | [34] |
| | Agar diffusion | Inhibitory | | 0.015% (v/v) (strong activity) | | [34] |
| *Cinnamomum camphora* (L) J. Presl. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| | Broth microdilution | Inhibitory | 3200–0.78 | 286.2 ± 27.9 μg/ml | 375.0 ± 34.8 | [36] |
| *Cinnamomum glandulifera* Nees. | Agar dilution | Inhibitory | | 700 μg/ml | | [40] |

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Cinnamomum zeylanicum* L. | Agar diffusion | Inhibitory | 2 mg/ml | | | [36, 59] |
| | Broth macrodilution | Inhibitory | | 58–83 μg/ml | 108–112 μg/ml | [42] |
| | | Inhibitory | | 25–100 mg/L | 25–100 mg/L | [45] |
| | | Inhibitory | | 38–50 μg/ml | | [46] |
| | | Inhibitory | | 25–67 μg/ml | | [6] |
| *Cinnamomum zeylanicum* + *Thymus vulgaris* L. | Broth macrodilution | Inhibitory | | 66.6 μg/ml | 95.83 μg/ml | [42] |
| *Citrus limon* L. | Broth microdilution | Inhibitory | | 764 mg/L | 2293 mg/L | [26] |
| *Cinnamomum zeylanicum* + *Thymus vulgaris* L. | Broth macrodilution | Inhibitory | | 66.6 μg/ml | 95.83 μg/ml | [42] |
| *Citrus limon* L. | Broth microdilution | Inhibitory | | 764 mg/L | 2293 mg/L | [42] |
| *Citrus nobilis* Lour | Broth microdilution | Inhibitory | | 815 mg/L | 2447 mg/L | [42] |
| *Citrus reticulata* var. *madurensis* Blanco | Agar diffusion | Inhibitory | 10 μl | | | [34] |
| | Agar dilution | Inhibitory | | 0.12–1.0% (v/v) | | [34] |
| *Copaifera officinalis* L. | Broth microdilution | Inhibitory | | 1.56% (v/v) | | [27] |
| *Copaifera officinalis* L. nanoemulsion | | Inhibitory | | 0.39% (v/v) | | [50] |
| *Cymbopogon citratus* + *Thymus vulgaris* L. | Agar dilution | Inhibitory | | 25–100 μg/ml | | [40] |
| *C. citratus* + *T. vulgaris* + *Satureja hortensis* L. + *Origanum vulgare* L. + *Ocimum basilicum* L. | Agar dilution | Inhibitory | | 25–175 μg/ml | | [40] |
| *C. citratus* + *T. vulgaris* + *O. basilicum* | Agar dilution | Inhibitory | | 50–350 μg/ml | | [40] |

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Cymbopogon martini* Stapf. | Broth microdilution | Inhibitory | | 1195 mg/L | 1208 mg/L | [42] |
| *Cymbopogon nardus* L. | Broth microdilution | Inhibitory | | 319 mg/L | 595 mg/L | [42] |
| *Daucus carota* L. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| | Broth microdilution | Inhibitory | 3200–0.78 μg/ml | 412.8 ± 26.0 μg/ml | 589.6 ± 48.2 μg/ml | [36] |
| *Eucalyptus cinerea* F. Muell | Agar diffusion | Inhibitory | 10 μl | | | [33] |
| *Eugenia* spp. | Agar diffusion | Inhibitory | | | | [32] |
| *Illicium verum* Hook.f. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| *Illicium verum* Hook.f. | Broth microdilution | Inhibitory | 3200–0.78 | 278.6 ± 21.2 μg/ml | 365.0 ± 32.1 | [36] |
| *Lavandula officinalis* L. | Broth macrodilution | Inhibitory | | 350–400 μg/ml | | [45] |
| *Laurus nobilis* L. | Broth microdilution | Inhibitory | | 1000 μg/ml | | [39] |
| | Broth microdilution | Inhibitory | | 12,879 μg/ml | | [36] |
| *Lepechinia floribunda* Benth. | Broth microdilution | Inhibitory | | 394 mg/L | 518 mg/L | [26] |
| *Lippia turbinata* Griseb | Broth macrodilution | Inhibitory | | 866 mg/L | | [26] |
| *Litsea cubeba* Pers. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| *Litsea cubeba* Pers. | Broth microdilution | Inhibitory | 3200–0.78 μg/ml | 85.0 ± 7.9 μg/ml | 186.0 ± 21.2 μg/ml | [36] |

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Melaleuca alternifolia* Maiden & Betche | Agar diffusion | Inhibitory | 10 µl | | | [34] |
| | Agar dilution | Inhibitory | | 0.015–0.12% (v/v) (strong activity) | | [34] |
| | Broth microdilution | Inhibitory | | 1095 mg/L | 1187 mg/L | [42] |
| | | Inhibitory | | 0.18–1.5% (v/v) | | [27] |
| | | Inhibitory | | 331 mg/L | 585 mg/L | [42] |
| | Broth microdilution | Inhibitory | | 1000–1800 µg/ml | 1600–2000 | [81] |
| *Mentha arvensis* L. | Broth microdilution | Inhibitory | | 144.7 ± 17.2 µg/ml | 248.0 ± 23.4 µg/ml | [36] |
| *Mentha* (hybrid) | Broth microdilution | Inhibitory | | 600–700 µg/ml | 1000–1200 µg/ml | [81] |
| *Mentha rotundifolia* L. | Broth microdilution | Inhibitory | | 600–1000 µg/ml | 1600 ≥ 2000 µg/ml | [81] |
| *Mentha spicata* L. | Agar diffusion | Inhibitory | 10 µl | | | [36] |
| | Broth microdilution | Inhibitory | 3200 to 0.78 µg/ml | 145.6 ± 15.4 µg/ml | 256.0 ± 26.5 µg/ml | [36] |
| *Minthostachys mollis* Kunth. | Broth macrodilution | Inhibitory | | 775 mg/L | | [42] |
| *Minthostachys verticillata* Griseb | Agar diffusion | Inhibitory | 10 µl | | | [33] |
| *Myristica fragrans* Gronov. | Agar diffusion | Inhibitory | 10 µl | | | [36] |
| *Myristica fragrans* Gronov. | Broth microdilution | Inhibitory | 3200–0.78 | 285.8 ± 29.2 µg/ml | 371.3 ± 29.0 | [36] |
| *Ocimum basilicum* L. | Agar dilution | Inhibitory | | 350–450 µg/ml | | [40] |
| | | Inhibitory | | 0.06–0.12% (v/v) | | [34] |

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Ocimum tenuiflorum* L. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| | Broth microdilution | Inhibitory | 3200–0.78 | 412.8 ± 26.0 μg/ml | 589.6 ± 48.2 | [36] |
| *Pimenta dioica* (L.) Merr. | Agar diffusion | Inhibitory | 10 μl | | | [36] |
| | Broth microdilution | Inhibitory | 3200–0.78 μg/ml | 78.0 ± 8.2 μg/ml | 162.0 ± 18.2 μg/ml | [36] |
| *Pimpinella anisum* L. | Agar diffusion | Inhibitory | 5 μl | | | [35] |
| | | Inhibitory | 10 μl | | | [35] |
| | Broth macrodilution | Inhibitory | | 300 μg/ml | | [46] |
| *Salvia officinalis* L. | Agar diffusion | Inhibitory | 5 μl | | | [35] |
| | | Inhibitory | 10 μl | | | [35] |
| *Salvia sclarea* L. | Agar diffusion | Inhibitory | 10 μl | | | [34] |
| | Agar dilution | Inhibitory | | 0.06% (v/v) (strong activity) | | [34] |
| *Satureja odora* Griseb. | Broth microdilution | Inhibitory | | 700–800 mg/L | 900 mg/L | [42] |
| *Schinus molle* L. | Broth macrodilution | Inhibitory | | 666 mg/L | | [42] |
| *Syzygium aromaticum* L. | Agar diffusion | Inhibitory | 10 μl | | | [34] |
| *Syzygium aromaticum* L. | Agar diffusion | Inhibitory | 5 μl | | | [35] |
| *Syzygium aromaticum* L. | Agar diffusion | Inhibitory | 10 μl | | | [35] |
| | Agar dilution | Inhibitory | | 0.015% (v/v) (strong activity) | | [34] |

| Essential oil | Technique | Activity | Amount tested | MIC[a] | MBC[b] | References |
|---|---|---|---|---|---|---|
| *Tagetes minuta* | Agar diffusion | Inhibitory | 10 µl | | | [34] |
| | Agar dilution | Inhibitory | | 500–650 µg/ml | | [40] |
| | Agar dilution | Inhibitory | | 700–800 µl/L | | [48] |
| | Broth macrodilution | Inhibitory | | 900–1000 mg/L | | [41] |
| | | Inhibitory | | 833 mg/L | | [42] |
| Thymol (component of *Thymus vulgaris*) | Broth macrodilution | Inhibitory | | 100–133 µg/ml | 133 µg/ml | [26] |
| *Trachyspermum ammi* L. | Agar diffusion | Inhibitory | 10 µl | | | [36] |
| | Broth macrodilution | Inhibitory | 3200–0.78 µg/ml | 137.0 ± 12.2 µg/ml | 224.8 ± 25.6 µg/ml | [36] |
| *Verbena officinalis* L. | Broth microdilution | Inhibitory | | 700–800 mg/L | 850 mg/L | [42] |
| *Wedelia glauca* Ortega | Broth microdilution | Inhibitory | | 700–800 mg/L | 950 mg/L | [42] |
| *Zingiber officinale* Rosc. | Agar diffusion | Inhibitory | 10 ml | | | [36] |

[a]*MIC, Minimal Inhibitory Concentration.*
[b]*MBC, Minimal Bactericidal Concentration.*

**Table 2.**
*Essential oils for the in vitro Paenibacillus larvae control.*

| Essential oil | Technique | Toxicity | Amount tested | References |
|---|---|---|---|---|
| *Carapa guaianensis* | Spraying procedure | Non-toxic | 25% (v/v) | [27] |
| *Carapa guaianensis* nanoemulsion | Complete exposure | Non-toxic | 10% (v/v) | [50] |
| | *In-vivo against larva* | Slightly toxic | | [50] |
| *Copaifera officinalis* | Spraying procedure | Non-toxic | 1.56% (v/v) | [27] |
| *Cymbopogon citratus* | Systemic administration | Moderately toxic (>2 μg EO/bee) | 1, 2, 4, 8, 16 and 32 μg EO/bee | [47] |
| *Cymbopogon citratus* + *Thymus vulgaris* (20:80, v/v) | Systemic administration | Slightly toxic (24 h-$LD_{50}$ = 15.94 μg b.e./bee) | 0.19, 0.37, 0.75, 1.50, 3.0 and 6.0 μg b.e./bee | [47] |
| *Cymbopogon citratus* + *Thymus vulgaris* + *Satureja hortensis* + *Origanum vulgare* + *Ocimum basilicum* (5:11:21:26:37, v/v/v/v/v) | Systemic administration | Not determined | 1.19, 2.37, 4.75, 9.50, 19.0 and 28.0 μg b.e./bee | [47] |
| *Cymbopogon citratus* + *Thymus vulgaris* + *Ocimum basilicum* (10:20:70, v/v/v) | Systemic administration | Virtually non-toxic (24 h-$LD_{50}$ = 122 μg b.e./bee) | 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 μg b.e./bee | [47] |
| *Cinnamomum zeylanicum* | Systemic administration | Virtually non-toxic | 2000, 4000, 8000 and 16,000 μg/ml | [46] |
| *Eucalyptus globulus* | Complete exposure | Non-toxic | 2.5, 5, 10 and 20 ml per cage of EO | [44] |
| *Eugenia* spp. | Systemic administration | Non-toxic | 400 μg/ml | [32] |
| *Melaleuca alternifolia* | Spraying procedure | Toxic/non-toxic the nanoparticles of *M. alternifolia* | 6.25% (w/v) | [49] |
| *Origanum vulgare* | Systemic administration | Moderately toxic (≥ 3 μg EO/bee) | 3, 6, 12, 24, 48 and 96 μg EO/bee | [47] |
| *Rosmarinus officinalis* | Complete exposure | Non-toxic | 2.5, 5, 10 and 20 μl per cage of EO | [51] |
| *Tagetes minuta* | Spraying procedure | Non-toxic | 5% (w/v) | [48] |
| *Thymus vulgaris* | Systemic administration | Moderately toxic (>8 μg EO/bee) | 2, 4, 8, 16, 32 and 64 μg EO/bee | [47] |

**Table 3.**
*Essential oils toxicity assays on Apis mellifera.*

*Pelargonium graveolens* L., were able to inhibit the growth of *P. larvae* by the agar diffusion technique [38–45].

EOs from *Cymbopogon citratus*, *Cinnamomum aromaticum*, *Citrus reticulata* var. madurensis, *Citrus paradisi*, *Heterothalamus alienus*, *Melaleuca alternifolia*, *Mentha piperita*, *Origanum majorana*, *Origanum vulgare*, *Salvia sclarea*, *Syzygium aromaticum*, *Tagetes minuta*, *Thymus vulgaris*, as well as the mixtures of *Cymbopogon citratus* and *Thymus vulgaris* EOs (20:80, v/v), and *Cymbopogon citratus*, *Thymus vulgaris*, *Satureja hortensis*, *Origanum vulgare*, and *Ocimum basilicum* EOs (5:11:21:26:37, v/v/v/v/v) showed antibacterial activity against *P. larvae* [44, 46, 47].

EOs from *Citrus sinensis*, *Cinnamomum* spp., *Eugenia* spp., *Thymus vulgaris*, *Verberna* spp., *Acantholippia seriphioides*, *Cinnamomum zeylanicum*, *Heterothalamus alienus* Spreng., *Pimpinella anisum*, *Foeniculum vulgare*, and *Eucalyptus globulosus*, and the mixture of *Thymus vulgaris* EO, thymol and *Cinnamomum zeylanicum* EO (62.5:25:12.5, v/v/v) exhibited antibacterial activity against *P. larvae* by the broth macrodilution technique [40, 48–52].

### *1.1.2 Toxicity assays on Apis mellifera*

*Citrus sinensis*, *Cinnamomum* spp., *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Eugenia* spp., *Thymus vulgaris*, and *Verbena* spp. EOs were non-toxic for adult honey bees when they were fed with candy and the EO at different concentrations by systemic administration [40, 53]. *Cymbopogon citratus*, *Thymus vulgaris* and *Ocimum basilicum* EOs, as well as *Cymbopogon citratus* and *Thymus vulgaris* EO mixture (50:50, v/v) were moderately toxic to adult honey bees. However, the *Cymbopogon citratus*, *Thymus vulgaris* and *Coriandrum sativum* EO mixture (33.3:33.3:33.3, v/v/v) presented negative mortality curves, meaning that there was less mortality at high doses. This fact disclosed that bees did not consume candy with high quantities of *Coriandrum sativum* EO [54]. When a solution containing a certain amount of EO was sprayed over a group of honey bees, *Tagetes minuta*, *Carapa guianensis* and *Carapa officinalis* EOs resulted to be non-toxic for adult bees [27, 55]; whereas *Melaleuca alternifolia* EO caused the death of the bees after 7 days of treatment. Nevertheless, the use of nanoparticles of *Melaleuca alternifolia* EO did not produce any toxic effect on honey bees [56]. *Eucalyptus globosus* and *Rosmarinus officinalis* EOs and the nanoemulsion of *Carapa officinalis* EO were not toxic for adult worker honey bees when they were completely exposed to the EO, that is, bees were in contact with the EO and ingested the EO [50, 57, 58]. The nanoemulsion of *Carapa guianensis* EO exhibited a toxic effect for larvae and adult honey bees, whereas the nanoemulsion of *Carapa officinalis* EO, a low toxic effect on larvae [57].

### *1.1.3 Mechanism of action of essential oils on P. larvae*

Different mechanisms of action of EOs on bacteria have been reported, among others: degradation of the cell wall, affecting the cell morphology and damaging the cytoplasmic membrane; damage of membrane protein, disruption of cell wall, leading to leakage of the cell contents, reduction of proton motive force, reduction of intracellular ATP pool, via decreasing ATP synthesis; inhibition of quorum sensing and alteration of cell division [59]. The alteration of the membrane permeability can be detected by the crystal violet assay [35] and the determination of the released UV-absorbing material assays [60]. The crystal violet assay is based on the fact that the compound enters easily when the cell membrane is defective. The released of UV-absorbing material assays is based on the fact that EOs can disrupt the cell membrane leading to a leakage of the cell content which is measured in the UV spectrum. The relationship between the chemical composition of EOs

and their antimicrobial mode of action against *P. larvae* has not been systematically researched so far. EOs are complex mixtures of low molecular weight volatile constituents biosynthesized by plants, which mainly include two biosynthetically related groups, i.e., terpenes and terpenoids, and aromatic and aliphatic constituents [61]. Most antimicrobial compounds are constitutively expressed by the plants, but others are synthesized as mechanism of defense in response to pathogens [59, 62]. Pellegrini et al. [62] demonstrated that the essential oils of *Acantholippia seriphioides*, *Aloysia polystachia*, *Buddleja globosa*, *Lippia turbinata*, *Minthostachys mollis*, *Schinus molle* and *Solidago chilensis* permeabilized and altered the cell membrane and the cytoplasmic membrane of *P. larvae* causing the leakage of cytoplasmic constituents.

### *1.1.4 Anti-quorum sensing and antimicrobial activity of essential oils*

Antúnez et al. (2010) [70] determined that during the division *P. larvae* produces and secretes different proteins with proteolytic activity, such as metalloproteases and enolase, these proteins are secreted and remain on the surface of the spores, producing a response in the immune system of *A. mellifera* and are probably involved in the degradation of larval tissue.

In recent years, the detection of quorum sensing (QS) detection signals in bacteria has added a new dimension to study the infection process. Through QS, bacteria depending on population density can activate specific genes [63–66]. The QS can regulate the expression of virulence factors, bioluminescence, sporulation, biofilm formation and conjugation [67–69]. Many bacteria coordinate the expression of multiple virulence factors, such as toxins, active redox compounds, siderophores, exoproteases, lipases and biofilm formation, thus maximizing the chances of infection and allowing better propagation [70, 71].

The QS signals occur while the bacterial population grows until it reaches a threshold concentration perceived by the bacteria and results in the activation or repression of specific genes. The accumulation of a stimulant amount of such molecules can occur only when a specific number of cells, known as a quorum, is present. These self-inducing molecules have been identified as acylated homoserine lactones in gram-negative and oligopeptide bacteria, thiolactone/lactone peptide, lanthionines, isoprenyl groups [65] and even acylated homoserine lactones in gram-positive bacteria [72, 73]. Similar signaling mechanisms have not yet been demonstrated in *P. larvae*. It is possible that larval infection by *P. larvae* is influenced by phenotypes regulated by QS, such as proteases exported by bacteria to their environment. The concept of QS has encouraged the development of a new non-antibiotic antibacterial therapy through the use of QS inhibitor compounds [74, 75].

The increase in resistance to multiple drugs of the bacteria against traditional medicines drastically reduces the efficacy of conventional antibiotics. This multiple resistance is now recognized as a global problem [76]. Therefore, it is necessary to develop a new therapeutic strategy to prevent this type of multidrugging. A promising mechanism is to block cell-to-cell communication, establishing a strategy called quorum extinction [77]. Although traditional antimicrobial agents cause cell death of the pathogen, the use of systems that alter the QS sensors adopts a less aggressive strategy [78]. There are several sources of QS inhibitors (quorum quenchers), but so far the most diverse and abundant are derived from natural sources such as algae and plants. There are cases of QS inhibitors in bacteria, fungi, algae, bryozoans, corals, sponges [79], plant extracts [80], essential oils [42], compounds isolated from bacteria [81] and furanones, among others.

Essential oils extracted from plants, such as *Cymbopogon citratus*, *Cymbopogon martini*, *Rosmarinus officinalis*, *Mentha piperita*, *Pelargonium odoratissimum* and

*Negundo vitex*, and different products, such as citral, geraniol, thymol and the linalool, have been used to evaluate its protease inhibitory activity, constituting one of the virulence factors of bacteria that can be regulated by QS [82].

Pellegrini et al. [62] propose that the EO will act by inhibiting the production of proteases, inhibiting its transportation and secretion, inhibiting the detection of quorum or avoiding the loading of proteases. All extracellular bacterial proteases are synthesized as an inactive pre-proenzyme consisting of a signal peptide, a prosequence and a maturity sequence. The peptide functions as a signal for the translocation of the pre-proenzyme to the membrane. The pre-proenzyme is processed in the proenzyme by the peptidase signal. The accusation acts as a molecular chaperone that leads to a self-cleavage of the peptide bond that links the pro and mature sequences [83]. The EOs acted at some point in this regulatory mechanism. The inhibition of larval proteases by EO could be a form of therapeutic intervention; the blocking of bacterial virulence factors does not destroy or inhibit the growth of pathogenic bacteria. It is expected that this strategy will generate little pressure on the selection of bacteria and, therefore, could diminish the appearance of bacterial resistance and avoid the interruption of the microbiota of benefits in urticaria. In future investigations, it will be interesting to isolate and characterize automatically the potential autoinductors of *P. larvae* and study their relationship with protease regulation. EOs studies are promising to use EOs in hives with symptoms of Foulbrood for the control of damage caused by *P. larvae*.

## 2. Conclusion

The research carried out to study the *in vitro* and *in vivo* antimicrobial activity of essential oils against *P. larvae*, their toxicity in adult honey bees, as well as their mode of action (degradation of the cell wall, affecting cell morphology and damaging the cytoplasm membrane, coagulation of the cytoplasm, etc.) and anti-QS activity (inhibiting the production of proteases, inhibiting transportation and secretion of proteases, inhibiting the detection of quorum, etc.), has been thoroughly reviewed throughout this chapter. As far as honeybee larvae are the target of AFB disease, future research should focus on studying the effect of essential oils that are effective *in vitro* and non-toxic for adult honey bees on honeybee larvae. In addition, more studies are still needed on the distribution and effects of these natural products in hives, adult honey bees, larvae, honey, royal jelly and other bee products to understand the pharmacokinetics and pharmacodynamics within the hive. As well, research on the effectiveness of these natural antimicrobials in field conditions is imperative. Moreover, further studies should be conducted on the sporicidal properties of these natural substances to destroy spores of *P. larvae* for the prevention of AFB disease. And last but not least, the development of adequate delivery modes of the essential oils within the hives for *in vivo* treatment and prevention of the disease is another important issue that requires further research, to put these natural strategies into practice under true hive conditions.

IntechOpen

## Author details

Sandra Rosa Fuselli[1,2], Pablo Gimenez Martinez[1,3*], Giselle Fuentes[1], Rosa María Alonso-Salces[1,4] and Matías Maggi[1,4]

1 Facultad de Ciencias Exactas y Naturales, Centro de Investigación en Abejas Sociales (IIPROSAM) , Universidad Nacional de Mar del Plata, Mar del Plata, Argentina

2 Comisión Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina

3 Agencia Nacional de Promoción Científica y Tecnológica, Buenos Aires, Argentina

4 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

*Address all correspondence to: beedarian@gmail.com

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

## References

[1] Buchmann S, Mirocha P. The Forgotten Pollinators. Island Press; 1996

[2] Klein AM et al. Wild pollination services to California almond rely on semi-natural habitat. Journal of Applied Ecology. 2012;**49**(3):723-732

[3] Le Conte Y, Ellis M, Ritter W. Varroa mites and honey bee health: Can *Varroa* explain part of the colony losses? Apidologie. 2010;**41**(3):353-363

[4] Neumann P, Carreck NL. Honey bee colony losses. Journal of Apicultural Research. 2010;**49**(1):1-6

[5] Potts SG et al. Global pollinator declines: Trends, impacts and drivers. Trends in Ecology & Evolution. 2010;**25**(6):345-353

[6] Genersch E. American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. Journal of Invertebrate Pathology. 2010;**103**(Suppl. 1):S10-S19

[7] Djukic M et al. How to kill the honey bee larva: Genomic potential and virulence mechanisms of *Paenibacillus larvae*. PLoS ONE. 2014;**9**(3):e90914

[8] Genersch E et al. Reclassification of *Paenibacillus larvae* subsp. pulvifaciens and *Paenibacillus larvae* subsp. larvae as *Paenibacillus larvae* without subspecies differentiation. International Journal of Systematic and Evolutionary Microbiology. 2006;**56**(3):501-511

[9] Ashiralieva A, Genersch E. Reclassification, genotypes and virulence of *Paenibacillus larvae*, the etiological agent of American foulbrood in honeybees—A review. Apidologie. 2006;**37**(4):411-420

[10] Bamrick JF. Resistance to American foulbrood in honey bees: VI. Spore germination in larvae of different ages. Journal of Invertebrate Pathology. 1967;**9**(1):30-34

[11] Genersch E, Ashiralieva A, Fries I. Strain- and genotype-specific differences in virulence of *Paenibacillus larvae* subsp. larvae, a bacterial pathogen causing American foulbrood disease in honeybees. Applied and Environmental Microbiology. 2005;**71**(11):7551-7555

[12] Hansen H, Brødsgaard CJ. American foulbrood: A review of its biology, diagnosis and control. Bee World. 1999;**80**(1):5-23

[13] Cornman RS, Lopez D, Evans JD. Transcriptional response of honey bee larvae infected with the bacterial pathogen *Paenibacillus larvae*. PLoS ONE. 2013;**8**(6):e65424

[14] De Graaf D et al. Diagnosis of American foulbrood in honey bees: A synthesis and proposed analytical protocols. Letters in Applied Microbiology. 2006;**43**(6):583-590

[15] Alippi A. Characterization of *Bacillus larvae* White, the causative agent of American foulbrood of honey-bees. First record of its occurrence in Argentina. Revista Argentina de Microbiología. 1992;**24**:67-72

[16] Alippi A. Characterization of isolates of *Paenibacillus larvae* with biochemical type and oxytetracycline resistance. Revista Argentina de Microbiología. 1996;**28**:197-203

[17] Marcangeli J. Pograma sanitario apicola del Partido de Mar Chiquita. Relevamiento de las enfermedades de abejas. In: XVII Congreso Latinoamericano de Parasitologia; Mar del Plata; 2005

[18] Servicio Agrícola Ganadero (SAG). Informe final del brote de Loque Americana en Chile. 2006. Available from: www.sag.cl/sites/default/file s/INFORME_FINAL_BROTE_OCTUBRE_2005.PDF

[19] Martel AC et al. Tetracycline residues in honey after hive treatment. Food Additives and Contaminants. 2006;**23**(3):265-273

[20] Miyagi T et al. Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. Journal of Invertebrate Pathology. 2000;**75**(1):95-96

[21] Harriet J, Campa J, Mendoza Y. Loque Americana: Cartilla No 22; 2013. Available from: http://www.ainfo.inia.uy/digital/bitstream/item/3821/1/Cartilla-22.pdf

[22] Piccini C, Zunino P. American foulbrood in Uruguay: Isolation of *Paenibacillus larvae* larvae from larvae with clinical symptoms and adult honeybees and susceptibility to oxytetracycline. Journal of Invertebrate Pathology. 2001;**78**:176-177

[23] Mutinelli F. European legislation governing the authorization of veterinary medicinal products with particular reference to the use of drugs for the control of honey bee diseases. Apiacta. 2003;**38**:156-168

[24] Lewis K, Ausubel FM. Prospects for plant-derived antibacterials. Nature Biotechnology. 2006;**24**(12):1504

[25] Damiani N et al. Laurel leaf extracts for honeybee pest and disease management: Antimicrobial, microsporicidal, and acaricidal activity. Parasitology Research. 2013;**113**:701-709

[26] Fuselli S et al. Inhibition of *Paenibacillus larvae* employing a mixture of essential oils and thymol. Revista Argentina de Microbiología. 2006;**38**(2):89-92

[27] Santos RCV et al. Antimicrobial activity of Amazonian oils against *Paenibacillus* species. Journal of Invertebrate Pathology. 2012;**109**(3):265-268

[28] Bastos EMA et al. *In vitro* study of the antimicrobial activity of Brazilian propolis against *Paenibacillus larvae*. Journal of Invertebrate Pathology. 2008;**97**(3):273-281

[29] Auddy B et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology. 2003;**84**(2-3):131-138

[30] Costa DC et al. Advances in phenolic compounds analysis of aromatic plants and their potential applications. Trends in Food Science & Technology. 2015;**45**(2):336-354

[31] Astani A, Reichling J, Schnitzler P. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2010;**24**(5):673-679

[32] Safaei-Ghomi J, Ahd AA. Antimicrobial and antifungal properties of the essential oil and methanol extracts of *Eucalyptus largiflorens* and *Eucalyptus intertexta*. Pharmacognosy Magazine. 2010;**6**(23):172

[33] Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology. 2004;**94**(3):223-253

[34] Kaloustian J et al. Étude de six huiles essentielles: Composition chimique et activité antibactérienne. Phytothérapie. 2008;**6**(3):160-164

[35] Devi KP et al. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. Journal of Ethnopharmacology. 2010;**130**(1):107-115

[36] Djenane D et al. Perspectives on the use of essential oils as antimicrobials against *Campylobacter jejuni* CECT 7572 in retail chicken meats packaged in microaerobic atmosphere. Journal of Food Safety. 2012;**32**(1):37-47

[37] Benjilali B et al. Méthode d'étude des propriétés antiseptiques des huiles essentielles par contact direct en milieu gélosé. Plantes Médicinales et Phytothérapie. 1986;**20**(2):155-167

[38] Ansari MJ et al. *In vitro* evaluation of the effects of some plant essential oils on *Paenibacillus larvae*, the causative agent of American foulbrood. Biotechnology & Biotechnological Equipment. 2016;**30**(1):49-55

[39] Fernández NJ et al. *Laurus nobilis* L. extracts against *Paenibacillus larvae*: Antimicrobial activity, antioxidant capacity, hygienic behavior and colony strength. Saudi Journal of Biological Sciences. 2018;**26**:906-912

[40] Floris I, Carta C, Moretti M. Activity of various essential oils against *Bacillus larvae* white *in vitro* and in apiary trials [antimicrobial actions, American foulbrood control]. Apidologie (France). 1996;**27**:111-119

[41] Gonzalez M, Marioli J. Antibacterial activity of water extracts and essential oils of various aromatic plants against *Paenibacillus larvae*, the causative agent of American foulbrood. Journal of Invertebrate Pathology. 2010;**104**(3):209-213

[42] Khan SU et al. Antimicrobial potentials of medicinal plant's extract and their derived silver nanoparticles: A focus on honey bee pathogen. Saudi Journal of Biological Sciences. 2018. In press

[43] Kuzyšinová K et al. Testing of inhibition activity of essential oils against *Paenibacillus larvae*—The causative agent of American foulbrood. Acta Veterinaria Brno. 2014;**83**(1):9-12

[44] Roussenova N. Antibacterial activity of essential oils against the etiological agent of American foulbrood disease (*Paenibacillus larvae*). Bulgarian Journal of Veterinary Medicine. 2011;**14**(1):17-24

[45] Tutun H, Koç N, Kart A. Plant essential oils used against some bee diseases. Turkish Journal of Agriculture-Food Science and Technology. 2018;**6**(1):34-45

[46] Alippi A et al. Actividad antimicrobiana *in vitro* de algunos aceites esenciales y mezclas de esencias sobre *Paenibacillus larvae* subsp. larvae. Vida Apícola. 2001;**106**:41-44

[47] Alippi AM et al. Antimicrobial activity of some essential oils against *Paenibacillus larvae*, the causal agent of American foulbrood disease. Journal of Herbs Spices & Medicinal Plants. 1996;**4**(2):9-16

[48] Fuselli S et al. Inhibition of *Paenibacillus larvae* subsp. larvae by the essential oils of two wild plants and their emulsifying agents. Spanish Journal of Agricultural Research. 2005;**3**(2):220-224

[49] Fuselli SR et al. Antimicrobial activity of some Argentinean wild plant essential oils against *Paenibacillus larvae* larvae, causal agent of American foulbrood (AFB). Journal of Apicultural Research. 2006;**45**(1):2-7

[50] Gende LB et al. Susceptibility of *Paenibacillus larvae* isolates to

a tetracycline hydrochloride and cinnamon (*Cinnamomum zeylanicum*) essential oil mixture. Bulletin of Insectology. 2010;**63**(2):247-250

[51] Gende LB et al. Antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*) essential oil and its main components against *Paenibacillus larvae* from Argentine. Bulletin of insectology. 2008;**61**(1):1

[52] Ruffinengo SR et al. Laboratory evaluation of *Heterothalamus alienus* essential oil against different pests of *Apis mellifera*. Journal of Essential Oil Research. 2006;**18**(6):704-707

[53] Gende LB et al. Antimicrobial activity of *Pimpinella anisum* and *Foeniculum vulgare* essential oils against *Paenibacillus larvae*. Journal of Essential Oil Research. 2009;**21**(1):91-93

[54] Albo G et al. Toxicidad de aceites esenciales con efecto fungistático sobre *Ascosphaera apis* en larvas y adultos de. Apis mellifera. 2008;**1**(7):1-7

[55] Eguaras MJ et al. An *in vitro* evaluation of Tagetes minuta essential oil for the control of the honeybee pathogens *Paenibacillus larvae* and *Ascosphaera apis*, and the parasitic mite *Varroa destructor*. Journal of Essential Oil Research. 2005;**17**(3):336-340

[56] Santos RCV et al. Antimicrobial activity of tea tree oil nanoparticles against American and European foulbrood diseases agents. Journal of Asia-Pacific Entomology. 2014;**17**(3):343-347

[57] de Almeida Vaucher R et al. Antimicrobial activity of nanostructured Amazonian oils against *Paenibacillus* species and their toxicity on larvae and adult worker bees. Journal of Asia-Pacific Entomology. 2015;**18**(2):205-210

[58] Maggi M et al. Bioactivity of *Rosmarinus officinalis* essential oils against *Apis mellifera*, *Varroa destructor* and *Paenibacillus larvae* related to the drying treatment of the plant material. Natural Product Research. 2011;**25**(4):397-406

[59] Nazzaro F, Fratianni F, Coppola R. Quorum sensing and phytochemicals. International Journal of Molecular Sciences. 2013;**14**(6):12607-12619

[60] Zhou K et al. Mode of action of pentocin 31-1: An antilisteria bacteriocin produced by *Lactobacillus pentosus* from Chinese traditional ham. Food Control. 2008;**19**(8):817-822

[61] Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;**17**(4):3989-4006

[62] Pellegrini MC et al. Inhibitory action of essential oils against proteases activity of *Paenibacillus larvae*, the etiological agent of American foulbrood disease. Spanish Journal of Agricultural Research. 2018;**15**(4):0504

[63] Henke JM, Bassler BL. Bacterial social engagements. Trends in Cell Biology. 2004;**14**(11):648-656

[64] Kleerebezem M et al. Quorum sensing by peptide pheromones and two-component signal-transduction systems in gram-positive bacteria. Molecular Microbiology. 1997;**24**(5):895-904

[65] Waters CM, Bassler BL. Quorum sensing: Cell-to-cell communication in bacteria. Annual Review of Cell and Developmental Biology. 2005;**21**:319-346

[66] Fuqua C, Parsek MR, Greenberg EP. Regulation of gene expression by cell to cell communication: Acyl homoserine lactone quorum sensing. Annual Review of Genetics. 2001;**35**(1):439-468

[67] Boyer M, Wisniewski-Dyé F. Cell-cell signalling in bacteria: Not simply a matter of quorum. FEMS Microbiology Ecology. 2009;**70**(1):1-19

[68] Cámara M, Williams P, Hardman A. Controlling infection by tuning in and turning down the volume of bacterial small-talk. The Lancet Infectious Diseases. 2002;**2**(11):667-676

[69] Williams P. Quorum sensing, communication and cross-kingdom signalling in the bacterial world. Microbiology. 2007;**153**(12):3923-3938

[70] Antúnez K et al. Secreted and immunogenic proteins produced by the honeybee bacterial pathogen, *Paenibacillus larvae*. Veterinary Microbiology. 2010;**141**(3-4):385-389

[71] Castillo-Juárez I et al. Role of quorum sensing in bacterial infections. World Journal of Clinical Cases: WJCC. 2015;**3**(7):575

[72] Biswa P, Doble M. Production of acylated homoserine lactone by Gram-positive bacteria isolated from marine water. FEMS Microbiology Letters. 2013;**343**(1):34-41

[73] Monnet V, Gardan R. Quorum-sensing regulators in Gram-positive bacteria: 'cherchez le peptide'. Molecular Microbiology. 2015;**97**(2):181-184

[74] Hentzer M et al. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. The EMBO Journal. 2003;**22**(15):3803-3815

[75] Rasmussen TB, Givskov M. Quorum-sensing inhibitors as anti-pathogenic drugs. International Journal of Medical Microbiology. 2006;**296**(2-3):149-161

[76] Olivero-Verbel J et al. Composition, anti-quorum sensing and antimicrobial activity of essential oils from *Lippia alba*. Brazilian Journal of Microbiology. 2014;**45**(3):759-767

[77] Zhang L-H. Quorum quenching and proactive host defense. Trends in Plant Science. 2003;**8**(5):238-244

[78] Otero Casal A et al. Quorum Sensing: El Lenguaje de las Bacterias. Zaragoza: Acribia, SA; 2005

[79] Tang K, Zhang X-H. Quorum quenching agents: Resources for antivirulence therapy. Marine Drugs. 2014;**12**(6):3245-3282

[80] Gala V, Desai K. Plant based quorum sensing inhibitors of *Pseudomonas aeruginosa*. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;**6**(8):20-25

[81] Chu Y-Y et al. A new class of quorum quenching molecules from *Staphylococcus* species affects communication and growth of gram-negative bacteria. PLoS Pathogens. 2013;**9**(9):e1003654

[82] Sivamani P et al. Comparative molecular docking analysis of essential oil constituents as elastase inhibitors. Bioinformation. 2012;**8**(10):457

[83] Inouye K et al. Engineering, expression, purification, and production of recombinant thermolysin. Biotechnology Annual Review. 2007;**13**:43-64

[84] OIE. World Animal Health Information Database - Version: 1.4. World Animal Health Information Database. Paris, France: World Organisation for Animal Health; 2009. Available from: http://www.oie.int